



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification <sup>4</sup> :</b> <b>A61K 9/16, 47/00, 49/00</b> <b>G01N 33/553</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 89/ 03674</b> <b>(43) International Publication Date:</b> <b>5 May 1989 (05.05.89)</b>
<b>(21) International Application Number:</b> PCT/SE88/00560 <b>(22) International Filing Date:</b> 24 October 1988 (24.10.88) <b>(31) Priority Application Number:</b> 8704158-8 <b>(32) Priority Date:</b> 26 October 1987 (26.10.87) <b>(33) Priority Country:</b> SE  <b>(71) Applicant (for all designated States except US):</b> CAR-BOMATRIX AB [SE/SE]; Soprangränden 15, S-223 68 Lund (SE).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only) :</b> SCHRÖDER, Ulf [SE/SE]; Soprangränden 15, S-223 68 Lund (SE). NY-BERG, Gunilla [SE/SE]; Spolegatan 18, S-222 20 Lund (SE). LAGER-UDEN, Catharina [SE/SE]; Agardsgatan 5, S-223 51 Lund (SE).		<b>(81) Designated States:</b> AT (European patent), BE (European patent), CH (European patent), DE (European patent), FR (European patent), GB (European patent), IT (European patent), JP, LU (European patent), NL (European patent), SE (European patent), US.  <b>Published</b> <i>With international search report.</i> <i>In English translation (filed in Swedish).</i>
<b>(54) Title:</b> MICROSPHERES, WAY OF PRODUCING SAID MICROSPHERES AND THE USE THEREOF  <b>(57) Abstract</b>  The present invention relates to a way of producing covalently crosslinked microspheres using biologically acceptable polymers and the use of these microspheres.		

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# MICROSPHERES, WAY OF PRODUCING SAID MICROSPHERES AND THE USE THEREOF.

This invention relates to a method for fabrication of covalently cross-linked microspheres where the matrix material consists of polymers, stabilized into a three dimensional network by crosslinking.

Microspheres according to this invention may be used within medical diagnostics, cell separation, drug targeting or as slow release systems for drugs. Within some of these areas a magnetically responsive material may be entrapped into the microspheres.

Microspheres or particles having a size of a few micrometers has been used for more than 60 years for studies within medical diagnostics, in particular in studying the reticuloendothelial functions. Thus, particles with associated radioactive substances has been used to study the liver, spleen, bone marrow and the lymphatic system and in investigations of the gastrointestinal tract, control of catheters and clinically in the treatment of certain tumors.

Furthermore, there is an expanding interest in producing microspheres with entrapped X-ray contrast agents for the use in diagnostics. The goal is to achieve a microsphere having a size of 2  $\mu\text{m}$  which may be injected into the blood stream. Such a microsphere would thus be useable as a specific contrast agent for the reticuloendothelial system (RES). The primarily interest is in obtaining a microsphere that could be used as a contrast agent for the liver.

Microspheres, as discussed in this invention, may also be used within cell biology. After associating the microsphere with a fluorescent dye, or any other detectable substance, and then on the surface attach an antibody, it is possible to use the microspheres in the analysis of a certain cell type in a heterogeneous mixture of cells.

The invention may also be used in the production and the use of slow release systems of biologically active substances taking advantage of a process that allows entrappment of the biologically active substance in a matrix system which is biodegradable and biocompatible. After injection to e.g. a human being, the entrapped substance is released in a predetermined way. The biologically active substances may be hormones such as insulin,

growth hormone, calcitonin, ACTH or the like. Other substances can be normal drugs or antigens for the use in vaccination.

The use of such a pharmaceutical formulation can be found in human as well as in the veterinary medicine and in the agricultural areas.

The terms "pharmaceutical substance" and "drug" are used in its broadest sense, such as a biologically active substance which has an effect and/or is used within the areas mentioned above.

Within the medical area it is possible to classify the biologically active substances according to their area of action.

Substances used for disorders in the lungs: cough relaxation (e.g. noskapin) or opiates (e.g. ethylmorphine), drugs reducing swelling of the mucus membrane (e.g. ephedrin, terbutalin, theophylline).

Substances for the heart or circulation; glycosides such as digoxin, lidocaine and procainamide.

Beta-blocking agents such as alprenolol or metoprolol. Other groups includes alfa-blocking agents (e.g. phentolamine), beta-stimulators (e.g. bametan), alkyl nitrates, calcium antagonists (e.g. nifedepin), derivatives of nicotinic acid, adrenergic compounds (e.g. adrenalin), sympaticus relaxatives (e.g. guanetidin), ganglieblockers (e.g. trimetaphan), hydrazinderivatives, tiaziderivatives, bensensulfonamidederivatives, bumetamid, furosemid, ethacrynic acid, spironlacton.

Varix treatment (e.g. polidokanol), cholesterol synthesis blockers (e.g. clofibrate).

Antihistaminic drugs (e.g. terbutalin) at allergic disorders.

Spasmolytic drugs; papaverinderivatives, anticholinergic drugs (e.g. atropin), cholinergic drugs (e.g. carbacol).

Drugs for tumor treatment; vitamins (e.g. B-12 or folic acid), alkylating substances (e.g. cyclofosfamide), antimetabolites (e.g. metotrexate or 5-fluorouracil), antibiotics (e.g. daunomycin, bleomycin), mitos blockers (e.g. vinblastin), cisplatinum, nitrousurea derivatives, estramustin, steroid-derivatives, cimetidin.

Chemotherapeutic and antibiotic substances; sulfonamides, penicillines, cephalosporines, tetracyclines, aminoglycosides, aminosalicic acid derivatives, isonicotinic acid derivatives, iodine.

Malaria drugs (e.g. chlorokin).

Drugs for the treatment of fungal infections. Vitamins.

Proteins or peptides; digestion enzymes, coagulation factors, immuno-

globulins, vaccines, hormones.

Immune stimulating substances (e.g. interferons, interleukins).

Various drugs used in psychiatric treatment.

Antiepileptic drugs and drugs used to obtain muscle relaxation.

Anticholinergic drugs, analgetic drugs and anesthetic drugs.

Thus, for the person skilled in the art it is obvious that these substances are not to any extent limited to the examples mentioned above, the substances can be, and has been, used in and with other indications than the above mentioned.

Within the agricultural area substances such as herbicides or pesticides may also be used.

A pharmaceutical formulation, such as described in this invention, is of particular interest, if it would be possible to produce a microsphere with entrapped biologically active substances, having a size that allows inhalation, e.g. formulated as a spray. The size of spheres should thus be 1-3  $\mu\text{m}$ . The use of such a preparation containing e.g. terbutalin<sup>®</sup> or theophylline, is seen in the treatment of asthma.

Another substance having a growing interest is nicotinic acid due to the positive effects seen in the treatment of certain tumors.

For the person skilled in the art, it is easy to apply and use the described invention to prepare formulations that would show a slow release effect of the entrapped biologically active substance.

Schematically, there are two basic methodologies to prepare a formulation for biologically active substances: entrapment or covalent attachment to a matrix. Furthermore, combinations of these technologies may be used. Using entrapment, one takes advantage of the properties of the matrix material and the biologically active substance to create association phenomena resulting in a stable preparation. Of great importance in the work with formulation in this area is that the degradation products produced by the matrix does not form or creates toxic metabolites. Making a choice regarding a suitable matrix material with this background, you are mainly restricted to the use of substances which are constituents of your own body and/or polymerized in biocompatible way.

One example of a polymers that may be used in the production of microspheres, and which are advantageous, are carbohydrate polymers containing  $\alpha(1 \rightarrow 4)$  bonds, such as starch, exhibit all the prerequisite for a

polymer to be used as a pharmaceutical formulation matrix for biologically active substances. Starch is a mixture of polymers where the monomers are glucose. The glucose monomers are linked together by  $\alpha(1 \rightarrow 4)$  bonds in the linear polymer chains. After fractionation of starch, a linear polymer called amylose, is obtained. Starch also shows a branched polymer called amylopectin, the bond at the branch point is a  $\alpha(1 \rightarrow 6)$  bond. Another carbohydrate polymer showing the same polymerization structure is glycogen. In vivo there are enzymes capable to degrade starch to glucose. This degradation in the body is performed by an enzyme called  $\alpha$ -amylase, showing specificity for  $\alpha(1 \rightarrow 4)$  bonds.

However, this invention is not restricted to the use of the above mentioned carbohydrates, although these are the preferred ones. Other types of carbohydrates, which easily may be applicated for the person skilled in the art, includes e.g. cellulose, chitosan, alginate, xantan, dextran, agarose, carrageenan, synthetic polymers or some derivative of these. Also included are guar gums, locust bean gum (galacto-mannan), gum arabicum, tragacanth gum, karaya gum as well as derivatives of these. Also heteropolysaccharides of glucose, galactose, mannose, glucuroic acid and fucose (Biopolymer PS-87, Lever Brothers Company, USA; US Patent 4,357,423) may be used according to this invention. These carbohydrate monomers are linked together by glycosidic bonds which may be hydrolyzed into smaller fragment which then may be excreted via the kidneys.

In the literature, a number of methodologies are described how to produce microspheres from various polymers. A few examples are mentioned below, all of which may be adopted to the present invention for the person skilled in the art.

As an example of phase evaporation, it may be noted that PLGA microspheres mainly are produced according to this technology (US Patent 4,389,930). Precipitation systems are described in PCT/SE83/00268 where the polymer used is starch. polymerization systems is described using starch (SE 7407461-8) or different kinds of microspheres based on acrylic polymers (J.Pharm.Sci. 1980, 69, 838-842). Also complexes and solutions are described (Swedish patent application 8501094.0) as useful formulations within this area.

In the production of a matrix material according to the present invention, a preformed polymer is used which is crosslinked. This separates

the present invention from the majority of technologies used for the production of microspheres where the starting material are the monomers which are linked together, forming a three dimensional network using various chemical reagents.

Since the present invention use preformed polymers, there is a need to introduce these branch points in order to obtain the three dimensional structure.

Examples of such a bifunctional reagents are epoxides (e.g. epichlorohydrine or various types of bisepoxides). This type of reaction takes advantage of the hydroxyl groups on the carbohydrate polymer and has been used in the known technology of producing carbohydrate microspheres (e.g. Sephadex<sup>®</sup>, Sepharose<sup>®</sup> and Spherex<sup>®</sup>). However, the epoxide crosslink is not regarded as a biodegradable bond. Furthermore, this type of crosslinking may not be used in the production of microspheres containing metal ions or metal oxides of the transition elements (e.g. Fe, Ni and Cr) since the epoxide primarily reacts with the Lewis acids formed, and not the carbohydrate.

The production of microspheres is primarily performed using emulsion technologies. This means, using carbohydrates, that the polymer is dissolved in an hydrophilic solvent (e.g. water), whereafter this solution is emulsified in a suitable hydrophobic phase which not is mixable with the carbohydrate solution. As a result, after mixing, small droplets of the carbohydrate solution is formed in the hydrophobic phase. However, in order to obtain a stable emulsion it is mostly necessary to add a surfactant. Such a substance has the ability of forming a stable boundary between the two phases. Schematically, such a surfactant has two surfaces - one hydrophilic and one hydrophobic - thus forming the stabilization layer between the two phases.

Of importance in the use of microspheres as a contrast agent or at inhalation (i.e. as a spray) is their ability to be suspendable as a monodispers suspension in a physiological water solution. This is conventionally performed using surfactants. However, using the present invention, a negatively charged microsphere is obtained which is optimal in order to avoid aggregation.

Using the present invention, which among other things discuss super-paramagnetic microspheres, there is a possibility to produce such microspheres in a simple and gentle procedure. Furthermore, their

application is described.

Thus, the invention also presents a procedure for the preparation of superparamagnetic microspheres which constitutes of a crosslinked biologically acceptable polymer into which a magnetically responsive material, preferably a metal oxide, has been entrapped. In this form the microspheres may be used as a contrast agent in medical diagnostics, in particular in Magnetic Resonance Tomography (MRT). It is also possible to use the microspheres as a matrix for biological molecules and thereby as a separation device. Further use for the microspheres is seen after combining the microspheres with drugs, after which the microspheres are injected into the blood stream and stopped at a predetermined site with the help of an external magnet (i.e. drug targeting).

Several magnetically responsive materials are available commercially and are described in the scientific literature. Preferentially used is however magnetite due to its cheap price, availability and stability in a water environment. Other materials that may be used includes inter alia Ni-NiO, Ni-TiO<sub>4</sub>, Mn-Fe ferrites, Ni, CoSm, Co, etc.

Using the present invention, superparamagnetic microspheres are obtained, indicating that they are not permanently magnetized when subjected to a magnetic field. Thus, the microspheres are easily resuspended into a monodispers suspension. Superparamagnetic microspheres are of utmost importance using the microspheres according to the present invention in order to avoid permanent aggregation

Magnetic microspheres has for long times been discussed as the most effective system in biotech separations (Hirschbein et al, Chemtech, March 1982, 172-179). However, prior art within this area lacks one or several prerequisites in order for the technology to gain acceptance on the market. The above mentioned superparamagnetic property is thereby one of the most important. Ithakissios (US Patent 4,115,534. Sept.19, 1978) describes magnetic polymer microspheres in the size range of 10-100  $\mu\text{m}$ , where the invention is exemplified using proteins or polyurethane as matrices with subsequent covalent crosslinking. Avrameas & Guesdon (US Patent 4,241,176. Dec.23, 1980) describes magnetic polymer microspheres where magnetite has been entrapped into a polyacrylamide-agarose spheres using an emulsion system and where the obtained microspheres have a size of 50-500  $\mu\text{m}$ . Widder et al (US Patent 4,230,685. Oct.28, 1980) describes a



process for the fabrication of microspheres where magnetic particles has been entrapped into albumin/protein-A with subsequent stabilization of the polymers. Mosbach (PCT/SE78/00001) describes adsorption of a prefabricated commercially available magnetite suspension (Ferrofluid®) into prefabricated agarose spheres (Sephacrose®). Ugelstad et al (PCT/NO83/00014) describes a preparation procedure where the magnetic material is precipitated inside prefabricated polymer microspheres of a defined size. Czerlinski (US Patent 4,454,234, June 12, 1984) describes magnetite particles which has been surface coated with a crosslinked polymer of acrylamide. Molday (US Patent 4,452,773, Jun.5, 1984) describes way of producing magnetite particles having a size of 10-70 nm, surface coated with a dextran polymer. Schröder & Borrebaeck (EPC 83901116.0) describes entrappment of magnetite particles into a carbohydrate matrix by an emulsion process with subsequent stabilization by crystallization of the carbohydrate polymer. Chagnon et al (EPO 0 125 995) describes a process for the preparation of magnetite particles which in a subsequent step are surface coated with a silicon polymer.

Another factor which has made the magnetic separation not gaining wide acceptance within large scale separation (e.g. fermentation) despite the patent and patent applications mentioned above, is that the microspheres, to be used in theses areas, must be simple to produce in large quantities combined with an equally simple way of attaching the affinity ligands.

At large scale separation there is a wish to separate e.g. human proteins produced by genetically engineered bacteria in fermentation tanks having a volume of, e.g. a thousand liter. In the separation step the superparamagnetic microspheres will aggregate. However, due to the superparamagnetic property the microspheres will immediately be suspended into a monodispers suspension upon switching off the magnetic field, thereby allowing the load of each microsphere to be recovered using known affinity technology. Another factor using the present invention is that due to the high polymer concentration used at the crosslinking, the affinity ligands are preferentially attached on the surface of the microsphere. This association of the ligands to the surface of microsphere renders a product that will consume smaller amounts of affinity ligand (e.g. a monoclonal antibody) thus making it more compatible. Furthermore, surface association renders fast adsorption of the desired substance due to the lack of diffusion barriers as

compared to Sepharose®, having a diameter above 50  $\mu\text{m}$  where most of the affinity ligands are inside the spheres.

Thus, the use of magnetic separation according to the present invention would result in the following advantages:

- the number of separation steps can be reduced
- higher yield
- faster process

These factors would consequently reduce the costs for separation and purification.

Using the process according to the present invention with the described superparamagnetic microspheres, there is now a possibility to achieve these goals.

Using superparamagnetic microspheres within the areas mentioned above, the size and the choice of polymer is of great importance.

Within medical diagnostics there is primarily a wish to obtain a contrast agent for the liver and the spleen. This may be achieved upon injection of microspheres having a size of 1-2  $\mu\text{m}$  into the blood stream since particles of this size are immediately eliminated from the circulation by these organs.

Using superparamagnetic microspheres within biotech separation it is also a wish to have microspheres in this range due to the high surface to volume ratio obtained. For example, reducing the size of a particle from 10 to 0.5  $\mu\text{m}$ , the surface available for attachment of affinity ligands is enhanced 20 times, as calculated on a defined volume packed beads.

However, the size should not be below 0,3  $\mu\text{m}$  since the magnetically responsive microsphere thereby contains too small amounts of magnetic material in order to be influenced by the magnetic field. Furthermore, with microspheres below this size particle/liquid interactions appear resulting in a suspension behaving more like a magnetic liquid thereby omitting the possibility of separating the discrete particles.

Another way of producing microspheres is by spray drying. However, these microspheres are not stable and has to further processed in order to be used in the areas discussed above. However, using the spray technology there is the possibility to introduce the crosslinking agent in the gas phase.

However, the invention is not restricted to the use of the mentioned methodologies for the different applications discussed above, the person skilled in the art may easily adopt the methods and use within other areas

additives except the carbohydrates described. This is obtained by precipitation of iron salts into an alkali solution where iron oxide is formed. However, this is performed by allowing both the iron salts and a solution of the starch, simultaneously to be added to the alkali solution while a powerful energy input is obtained by sonication.

The suspension obtained, containing a monodispers suspension of superparamagnetic particles in a neutral or alkali solution of carbohydrates, may now after concentration step, be emulsified in an organic solvent, a crosslinker may be added leading to a stabilization of the microdroplet suspension into a water insoluble three dimensional polymer network.

The following examples are to be considered as guidances and not to any extent limiting since the basic technologies used are well known to the person skilled in the art and may therefore easily be modified or changed without changing the essence of the present invention.

#### Example 1.

2.7 g  $\text{FeCl}_3 \times 7 \text{H}_2\text{O}$  and 4.5 g  $\text{FeCl}_2 \times 4 \text{H}_2\text{O}$  is dissolved together with 3.0 g of starch in 10 ml of water by gently heating. This solution is added to 100 ml of 1.0 M NaOH while sonicating. After adding all the iron chloride/starch the sonication is proceeded for another 5 minutes. Sodium trimetaphosphate is added to this suspension at a final concentration of 10 mg/ml whereafter the superparamagnetic particle suspension is emulsified in an organic solvent consisting of dichloroethane, containing the emulsifier Gafac PE-510®, at a final concentration of 2%. The ratio between the hydrophilic and the hydrophobic phase is 1:10. The emulsion is stirred in order to obtain the size of interest. After 60 minutes the emulsion is poured into 4 volumes of acetone, centrifuged and then washed another 5 times the same way. If the superparamagnetic microspheres are to be used for biotech separation, a tresyl activation is performed at this stage.

#### Example 2.

To 20 mg of suspended tresyl activated superparamagnetic microspheres according to example 1, radiolabelled bovine serum albumin was added and subsequently coupled to the microspheres. After 1.5 hours of incubation the spheres were washed extensively with the help of permanent magnets on the outside of the test tube. It was determined that 3.5  $\mu\text{g}$  of the

where there is a need of methodology producing a matrix system with the properties as described in the present invention.

## DESCRIPTION OF THE INVENTION.

One crosslinker which we have found to work in a carbohydrate system containing metals/metal oxides, is sodiumtrimetaphosphate (STP). This substance is well known as a crosslinker in the starch industry. However, it is not described how and in what way it could be used in order to produce microspheres in the way described and in the application areas as discussed in the present invention.

In the preparation of pharmaceutical formulations there is, as mentioned above, a requirement on the formulation to be biocompatible. Using STP as a crosslinker phosphate esters are created as bonds between the polymer chains. It has been shown that after degradation the phosphate bonds are attached to the C-6 (63%), C-2 (28%) and the C-3 (9%) (Wurzburg, Modified Starches, CRC Press 1986, page 104). All of these phosphate esters of the degraded starch into glucose, are normal constituents of the cell machinery and consequently metabolized within the body.

The combination of a biodegradable polymer (starch) and a biodegradable crosslinking (phosphate esters), should thus be an optimal pharmaceutical formulation.

The present invention relies on the covalent crosslinking of a carbohydrate solution in an emulsion or to perform the crosslinking in a partially water containing environment of prefabricated microspheres.

The process for fabrication of microspheres according to the present invention is based on the optimization of the parameters in order to achieve microspheres which all fulfill the requirements discussed above. The process, according to the present invention renders a high yield where secondary purification steps in order to achieve optimal microspheres having the adequate size is not necessary.

A prerequisite in producing the superparamagnetic microspheres according to the present invention, is that the magnetite particles to be entrapped into the three dimensional polymeric network can be obtained as a stable suspension in an alkali solution or at neutral pH without any

BSA was attached per mg of spheres.

Example 3.

In order to assess stability regarding size, the spheres were suspended in physiological water solution (phosphate buffered saline, pH 7.4). The size was measured daily with the help of a Coulter Counter Multisizer® for four consecutive days with the following result.

Day 1	2.38 $\mu\text{m}$
Day 2	2.24 $\mu\text{m}$
Day 3	2.20 $\mu\text{m}$
Day 4	2.38 $\mu\text{m}$

## CLAIMS

1. Microspheres, **characterized by**, that they include a biologically acceptable polymer matrix which is crosslinked by phosphate ester bonds.
2. Microspheres according to claim 1, **characterized by**, that they contain a magnetically responsive material.
3. Microspheres according to claim 1 and 2, **characterized by**, that the magnetically responsive material is superparamagnetic.
4. Microspheres according to one or several of the claims 1-3, **characterized by**, that the average diameter is in the range of 0.1 - 1000  $\mu\text{m}$ .
5. Microspheres according to one or several of the claims 1-4, **characterized by**, that the polymer matrix consists of a carbohydrate or a protein.
6. A way of producing the crosslinked microspheres according to one or several of the previous claims, **characterized by**, that a crosslinker, in the form a water soluble phosphate is added to a water solution of a biologically acceptable matrix material, whereafter the water solution is emulsified in a hydrophobic medium thus creating microspheres with phosphate ester crosslinks, or that the water soluble phosphate is added to a suspension of preformed microspheres prepared from a biologically acceptable polymer matrix thereby creating microspheres with phosphate ester crosslinks.
7. A way of producing microspheres according to claim 6, **characterized by**, that the crosslinker used is sodiumtrimetaphosphate.
8. The use of microspheres according to claims 1-7 in medical diagnostics, cell separation, pharmaceutical formulations as well as

within chemical and biological analysis.

# INTERNATIONAL SEARCH REPORT

International Application

PCT/SE88/00560

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) \*

According to International Patent Classification (IPC) or to both National Classification and IPC 4

A 61 K 9/16, 47/00, 49/00; G 01 N 33/553

## II. FIELDS SEARCHED

Minimum Documentation Searched 7

Classification System

Classification Symbols

IPC 4

A 61 K, G 01 N

Documentation Searched other than Minimum Documentation  
to the extent that such Documents are included in the Fields Searched \*

SE, NO, DK, FI classes as above

## III. DOCUMENTS CONSIDERED TO BE RELEVANT \*

Category *	Citation of Document, 11 with indication, where appropriate, of the relevant passages 12	Relevant to Claim No. 13
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A	EP, A2, 0 184 710 (BAYER AG) 18 June 1986 The whole document	1 - 8
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### \* Special categories of cited documents: 10

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"Z" document member of the same patent family

## IV. CERTIFICATION

Date of the Actual Completion of the International Search

1989-02-15

Date of Mailing of this International Search Report

1989 -02- 2 0

International Searching Authority

Swedish Patent Office

Signature of Authorized Officer

Solveig Gustavsson



## FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

(partially)

V. ☒ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE <sup>1</sup>

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☒ Claim numbers 8 because they relate to subject matter not required to be searched by this Authority, namely:

in the aspect when the claim concerns diagnostic methods of the human or animal body (see article 17 (2) and rule 39).

2. ☐ Claim numbers           , because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers           , because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING <sup>2</sup>

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the international Searching Authority did not invite payment of any additional fee.

## Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.